REMARKS

The present invention is directed to methods of inhibiting growth of a refractory tumor by administering an EGFR antagonist and either a chemotherapeutic agent and/or radiation. Claims 36-76 and 126-139 are currently pending, and claims 36-58 and 126-139 are currently under consideration.

Applicant would like to thank the Examiner, Anne Holleran, and her supervisor, Anthony Caputa, for the courtesy extended to their attorneys, Thomas C. Gallagher and Kathryn M. Lumb, in granting an Interview on March 7, 2003, to discuss the outstanding issues and to advance prosecution of the present application.

Discussion of Office Action

In an Office Action dated December 17, 2003, the Office (i) required restriction to either of two groups; (ii) rejected claims 48-50 as not enabled under 35 U.S.C. § 112, first paragraph; and (iii) rejected claims 36-47, 51-58, 74-76, and 126-127 as obvious under 35 U.S.C. § 103(a). In addition, the Office has withdrawn the restriction requirement of the Office Action of July 16, 2002, which applicant gratefully acknowledges.

Amendments to the Claims

In response to suggestions made during the Interview and in the interests of advancing prosecution of the present application, applicant has made various amendments to the claims. Claims 36, 127, and 128 have been amended to specify that the human to whom the EGFR antagonist and chemotherapy are administered has a refractory tumor that has failed or been resistant to treatment with an antineoplastic, such a chemotherapeutic agent, radiation, or a biological response modifier. *See, e.g.*, p. 5, ll. 22-23. Claims 48-50 have been amended to depend from claim 59, rather than claim 45. *Id.* at p. 14, l. 24-p. 16, l. 7. Claims 53 and 58 have been amended to recite an antibody, or fragment thereof. *Id.* at p. 9, ll. 1-15. Finally, claim 59 has been amended to specify that the small molecules of the

present invention can have a molecular weight of less than or about 450. *Id.* at p. 14, ll. 19-21.

In addition, applicant has amended or added various additional claims. Claims 65, 69, and 72 have been amended to correct typographical errors. New claims 128-132 have been added to recite that the chimeric antibody is administered at a loading dose of about 100, 400, or 500 mg/m^2 and a weekly dose of about 100 or 250 mg/ m^2, respectively. *See, e.g.*, p. 21, ll. 21-23. New claim 133 has been added to recite that the cisplatin is administered at a weekly dose of about 250 mg/m^2. *Id.* Claim 134 has been amended to recite that the EGFR-specific antibody is administered at a loading dose of about 400 mg/m^2. *Id.* at p. 23, ll. 12-18. New claims 135 and 136 have been added to recite that the EGFR-specific antibody is administered at a weekly dose of about 250 mg/m^2. *Id.* at p. 23, ll. 15-17. New claims 137-139 have been added to recite that the chemotherapeutic agent is administered at a dose of 125 mg/m^2 and that the EGFR antagonist is administered at a loading dose of about 400 mg/m^2 and a weekly dose of about 250 mg/m^2, respectively. *Id.*

Applicant submits that the amendments and new claims are fully supported by the specification, and no new matter is added.

Restriction Requirement

In the requirement for restriction set forth by the Office, applicant is required to elect one of the following groups of invention: (i) claims 36-76 and 126-127, which is drawn to methods of treatment comprising administering an EGFR antagonist and a chemotherapeutic agent, or (ii) claims 77-125, which is drawn to methods of treatment comprising administering an EGFR antagonist and radiation. Additionally, the Office requires election of a single species of EGFR antagonist. Applicant's attorney, Ms. Lumb, has provisionally elected, with traverse, the invention of group I and also the species of an EGFR antagonist to be an antibody.

In response, applicant affirms this election, with traverse. Applicant submits that claims 77-80 would more properly be examined with group I. However, in the interests of advancing prosecution of the present application, applicant has withdrawn claims 77-80 as well as claims 81-125 from consideration. Applicant reserves the right to file a divisional application directed to such non-elected subject matter.

Enablement Rejection

The Office alleges that claims 48-50 are not enabled. Applicant submits that claims 48-50 are clearly enabled and respectfully request that the rejection be withdrawn.

The specification teaches those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. While applicant believes that the claims as previously pending are fully enabled by the specification, claims 48-50 have been amended to depend from claim 59, rather than claim 45. As amended, these claims are now drawn to methods of administering a small molecule antagonist, rather than an antibody antagonist. The small molecule antagonist bind EGFR internally, inhibits binding of ATP to EGFR, or competes with ATP for EGFR. As discussed during the Interview, it is known that small molecule tyrosine kinase inhibitors function by binding internally to receptors and competing with the protein substrate or ATP for binding to the tyrosine kinase domain, thereby preventing both autophosphorylation of the receptor and phosphorylation of target proteins that function in the signal transduction cascade. *See, e.g.*, Baselga et al., Epidermal Growth Factor Receptor: Potential Target for Antitumor Agents, The Center for Biomedical Continuing Education, at Fig. 1 (attached hereto). As such, the present disclosure provides enablement commensurate in scope with the amended claims.

Obviousness Rejection

The Office alleges that claims 36-47 and 51-53 are obvious in light of Baselga et al. (*J. Nat'l Cancer Inst.*, 85: 1327-33 (1993); "Baselga (1993)"), Fan et al. (*Cancer Res.*, 53:

4637-42 (1993); "Fan") or Baselga et al. (*Breast Cancer Res. Treatment*, 29: 127-38 (1994); "Baselga (1994)") in view of Mendelsohn (U.S. Patent No. 4,943,533; "the '533 patent"). The Office also alleges that claims 36, 54-58 and 126 are obvious in light of Prewett et al. (*Int'l J. Oncology*, 9: 217-224 (1996)[1]) in view of Mateo de Acosta del Rio (U.S. Patent No. 5,891,996) or Bendig (U.S. Patent No. 5,558,864). The Office further alleges that claims 36, 74, and 127 are obvious over Baselga (1994), *supra*, in combination with Prewett, *supra*, and Punt (*Cancer*, 83:679-89 (1998)).

Applicant submits that the Office has not set forth a *prima facia* case of obviousness. To establish *prima facia* obviousness, *inter alia*, the prior art references, when combined, must teach or suggest all the claim limitations, there must be some suggestion or motivation to combine the reference teachings and a reasonable expectation of success. M.P.E.P. § 2143. The Office asserts with each rejection that it was *prima facia* obvious "to have developed a method for treatment of humans that comprised administering an anti[-]EGF[R] antibody and a chemotherapeutic agent." See Office Action, December 17, 2002 ¶ 12. However, as discussed during the Interview, the Office has overlooked the fact that the claims recite administration to a human having a refractory tumor that has failed or been resistant to treatment with an antineoplastic.

Taken together, none of the cited references teach or suggest the presently claimed treatment of tumors that are refractory to conventional therapies with a combination of an anti-EGFR antibody and an antineoplastic. Refractory tumors lead to rapid disease progression, usually with poor prognosis. *See, e.g.*, p. 4, l. 25-p. 5, l. 1. As described in the specification, refractory tumors are endogenous tumors, native to the human patient being treated. (*See, e.g.*, p. 6, ll. 9-10.) Treatment of human patients with refractory cancer that had previously been treated with a chemotherapeutic (e.g., cisplatin), radiation,

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[1] Although the Office has not specified which Prewett reference allegedly renders the claimed invention obvious, applicant has assumed that this is the reference as it was cited in the most recent IDS.

or a biological response modifier (e.g., Ad p53, which is a replication-deficient adenovirus delivering p53) is described in the specification in Example 1. (*Id.* at p. 23, ll. 3-10.)

In contrast, none of the references cited teach or suggest treatment of refractory tumors, specifically combination treatment with an EGFR antagonist and an antineoplastic. Baselga (1993) teaches that anti-EGFR antibodies are capable of enhancing the effect of doxorubicin against xenographs of a squamous cell carcinoma line and an adenocarcinoma cell line. *See, e.g.*, p. 1331. Fan teaches growth inhibition of tumor xenographs of an epidermoid cancer cell line following treatment with an anti-EGFR MAb in combination with a chemotherapeutic agent, *cis*-DDP. *See, e.g.*, p. 4639. Administration of anti-EGFR antibodies to human breast adenocarcinoma cell xenographs, see, e.g., p. 130, and in combination with doxorubicin to human breast adenocarcinoma cell xenographs and epidermoid cancer cell xenographs, see, e.g., p. 132, are taught by Baselga (1994) to result in marked inhibition of tumor growth. Futhermore, Baselga (1994) suggests use of a combination of anti-EGFR antibodies and chemotherapeutics or additional MAbs in xenographs of human stomach adenocarcinoma cells or breast carcinoma cells. *See, e.g.*, p. 134. Prewett teaches treatment of xenographic tumors (oral epidermoid carcinoma cells) in athymic nude mice with a chimeric anti-EGFR antibody and cisplatin. *See, e.g.*, p. 218, col. 2, ¶ 4.

The xenograph tumors taught by the cited references are not refractory tumors that have failed or been resistant to treatment with an antineoplastic according to the present claims. Following implantation of the tumor cell lines in the nude mice, none of the xenograph tumors were subjected to any treatment prior to administration of the EGFR antagonist and a chemotherapeutic agent. Thus, these xenograph tumors have not failed or been resistant to treatment with an antineoplastic. Moreover, the xenograph tumors disclosed are all implanted tumor cell lines and are not endogenous occurring in the animal, as opposed to the claimed refractory tumors of the present invention, which are

native to the animal. Accordingly, these cited references do not teach or suggest the recited refractory tumors of the presently claimed invention.

The rest of the references simply teach various monoclonal antibodies against EGFR or other chemotherapeutic agents, with no teaching or even mention of combination treatment of tumors refractory to conventional therapies. Mendelsohn teaches anti-EGFR MAbs 579, 455, 255, and 528, for example, and methods of production thereof. *See, e.g.*, col. 4, l. 1-col. 10, l. 47 (Examples 1-4). Humanized and chimeric anti-EGFR monoclonal antibodies are also taught by Mateo de Acosta del Rio, see, e.g., col. 10, ll. 17-52 (Example 4), and Bendig, see, e.g., col. 25, l. 20-col. 26, l. 57 (Example 4). Finally, Punt teaches various chemotherapeutic agents for the treatment of colorectal cancer, including irinotecan, see, e.g., p. 680, and oxaliplatin, see, e.g., p. 681, for example, with no discussion of EGFR at all.

In addition, even if the combined references were to teach or suggest treatment of refractory tumors, there is no reasonable expectation that such combination treatment would be successful. By definition, as recited in the claims as presently amended, refractory tumors have failed or been resistant to treatment with conventional therapies. Use of any other treatment is simply a guess as to whether or not it will be successful, and one skilled in the art would not be able to predict that any specific treatment regime would be effective until they had actually treated a patient with it. Accordingly, based on the teachings of the cited references taken together, there is no a reasonable expectation that combination therapy using an EGFR antagonist and an additional antineoplastic will be successful in treating refractory tumors that rises to the degree of predictability required for *prima facia* obviousness.

A *prima facia* case of obviousness thus has not been established. In light of these remarks, applicant respectfully requests that the obviousness rejections be withdrawn.

CONCLUSION

Applicant believes that the present application is in condition for allowance, and respectfully request that the Office pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

The Office is authorized to charge any fees that may be necessary for consideration of this paper to Kenyon & Kenyon Deposit Account No. 11-0600.

Respectfully submitted,

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Dated: May 6, 2003

Epidermal Growth Factor Receptor: Potential Target for Antitumor Agents

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Epidermal Growth Factor Receptor: Potential Target for Antitumor Agents

Date of Original Release: May 2000
Date of Expiration: May 2001

This CME activity was planned and produced in accordance with the ACCME Essential Areas and Policies by *The CBCE*™ (*Center for Biomedical Continuing Education*).

Objectives

After reading this monograph, physicians should be able to:

1. Discuss the in vitro and in vivo preclinical data on the role of the epidermal growth factor receptor in cancer.
2. Compare the degree of overexpression of the epidermal growth factor receptor in various human tumors.
3. Identify the issues surrounding the detection of the epidermal growth factor receptor in tumors.
4. Characterize the novel strategies aimed at inhibiting the epidermal growth factor receptor in human cancers, such as monoclonal antibodies and tyrosine kinase inhibitors.
5. Evaluate the current status of overexpression of the epidermal growth factor receptor as a prognostic factor in cancer.

Target Audience

Oncologists and immunologists interested in learning about new advances in biological therapy and new drugs in cancer.

Estimated Study Time for Completing This CME Activity

The estimated time for reading the monograph and completing the test is 2.5 hours.

Accreditation Statement

The CBCE is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

Designation Statement

The CBCE designates this educational activity for a maximum of 2.5 hours in category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those hours of credit that he/she actually spent on the educational activity.

Inquiries

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Cover: Photomicrograph of a section from a human colon adenocarcinoma exhibiting expression of epidermal growth factor receptor (EGFR). Expression was detected by immunohistochemical staining using a monoclonal anti-EGFR antibody, followed by a visualization system employing the dextran polymer technology and diaminobenzidine substrate. The brown color on the membrane reveals where the anti-EGFR antibody has bound, identifying tumor cells that express EGFR on the cell surface. (Photomicrograph courtesy of DAKO Corporation.)

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Contents

Introduction

The key role that growth factors and their receptors play in the development and progression of human cancers has been recognized for many years. Binding of growth factors to their cognate cell surface receptors induces receptor activation, which initiates or modifies signal transduction cascades. These growth-regulating molecules and their receptors act to modulate cell proliferation and differentiation in normal tissues as well as during embryogenesis. In many cancers, however, growth factors or their receptors are overexpressed or aberrantly expressed. Such abnormal stimulation of growth factor pathways results in unregulated cell signaling, contributing to dysregulation of growth, tumor initiation or promotion, and metastasis. The degree to which tumors capitalize on these cell signaling pathways is illustrated by the large number of oncogenes related to cellular genes encoding growth factors (e.g., *sis, hst*), growth factor receptors (*kit, trk, erbB-2*), or tyrosine kinases (*abl, src, lck*).[1]

The epidermal growth factor receptor (EGFR) gene encodes a 170-kD membrane-spanning glycoprotein composed of an extracellular ligand binding domain, a transmembrane region, and a cytoplasmic protein tyrosine kinase domain.[2] The EGFR is thought to play an important role in the regulation of cell division and tumor growth. Binding of specific ligands such as EGF to the extracellular domain results in EGFR autophosphorylation, activation of the receptor's cytoplasmic tyrosine kinase domain, and initiation of multiple signal transduction pathways that regulate tumor cell growth and survival (Figure 1). As is the case with other growth factor receptors, increased EGFR activation can result from higher levels of ligand (such as EGF), EGFR gene amplification, increased transcription, or mutations that cause unregulated receptor signaling. In many cancers this excessive EGFR activation has been shown to stimulate tumor growth. Preclinical studies indicate that EGFR-mediated signaling may also affect other aspects of tumor progression including angiogenesis, cell survival, and metastasis. In human cancers, such phenotypic changes appear to be critical for disease progression and patient survival.

The EGFR is expressed on the cell surface in many normal tissues, and elevated numbers of these receptors have been detected on a variety of human tumors including breast,[3,4] lung,[5] head and neck squamous cell carcinomas,[5,6] glioblastoma multiforme,[7,8] and colorectal carcinomas.[5,9] Several studies have demonstrated that overexpression of EGFR in various tumor types is associated with a poor patient prognosis.[10-14] A better understanding of the biology of the EFGR and its role in tumor progression is therefore critical to the development of new antitumor therapeutics that target the EGFR and inhibit its function.

Epidermal Growth Factor Receptor Ligands

Growth factor receptors such as platelet-derived growth factor receptor (PDGFR) and EGFR generally bind one or more growth factor proteins or other molecules, which regulate the activity of the receptor. Ligands for EGFR include EGF, transforming growth factor alpha (TGF-α), amphiregulin, heparin-binding EGF (HB-EGF), and betacellulin.[3] EGF and TGF-α are believed to be the main endogenous ligands that result in EGFR-mediated stimulation, although TGF-α has been shown to be more potent in promoting angiogenesis.[15] These ligands bind to and activate EGFR, resulting in receptor dimerization and autophosphorylation. Over-expression of growth factor receptors (e.g., EGFR, or HER2 in some metastatic breast cancers) can make tumor cells more sensitive to low concentrations of growth factors.[2]

These growth factors are expressed in a variety of tissues and act to stimulate EGFR-mediated growth and/or differentiation in normal and tumor cells. The ligands can be secreted from tumor cells in an autocrine manner where they are rapidly bound by their growth factor receptors on the cell surface. In fact, many tumor cells require continued stimulation by specific growth factors for cell survival and proliferation. Overproduction of growth factors (e.g., by expression vectors) can result in the transformation of various cell types in vitro or in vivo.[16] Consequently, antibod-

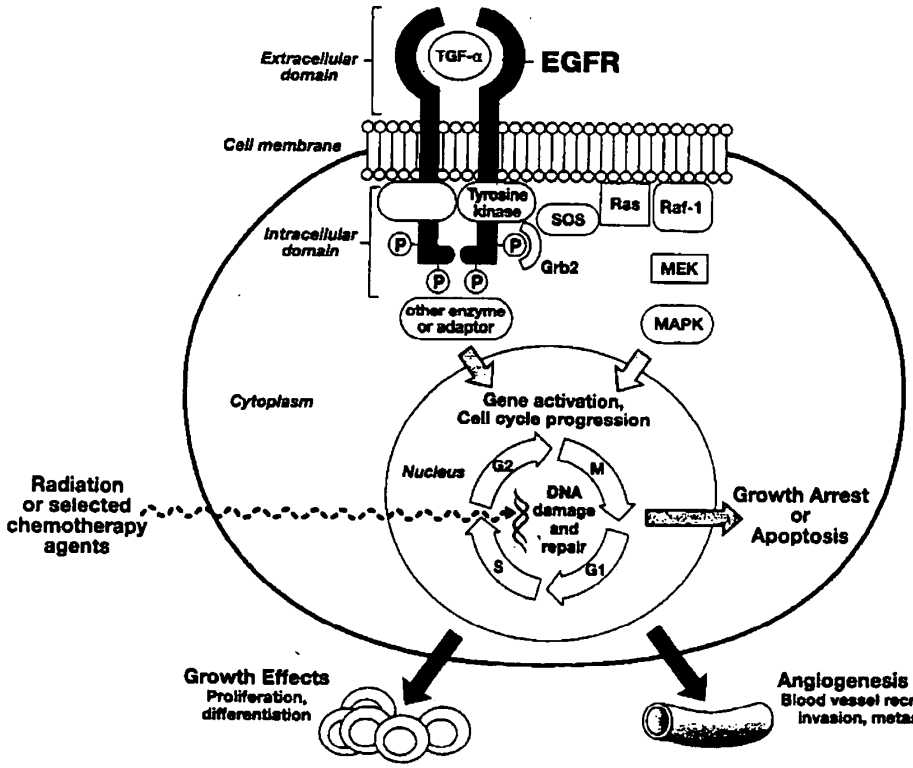
ies that block ligand binding to these receptors can prevent EGFR-mediated stimulation and trigger apoptosis.

Epidermal Growth Factor Receptor

The EGFR is one of four known related members of a family of growth factor receptors that are important mediators of cell growth, differentiation, and survival. This receptor family is composed of HER1 (EGFR or ErbB1), HER2 (*neu* or ErbB2), HER3 (ErbB3), and HER4 (ErbB4); some of these have intrinsic tyrosine kinase activity.[1,7] Signaling by the EGFR protein is normally mediated through binding of ligand, which results in EGFR homodimerization or heterodimerization with another member of the receptor family such as HER2, receptor autophosphorylation, and activation of the EGFR tyrosine kinase (Figure 1). This results in signal transduction through multiple intracellular pathways, leading to cell proliferation and other key events that can affect tumor progression.[2]

Enhanced activity or overexpression of EGFR has been associated with tumor progression in a number of different types of studies. First, overexpression of EGFR has been observed in a variety of human cancers, including brain (40%-50% of glioblastoma multiforme),[7,8] breast (range,

Figure 1. Schematic of the Epidermal Growth Factor Receptor and its Role in Signal Transduction and Tumor Progression



Simplified diagram of the EGFR pathway consisting of the epidermal growth factor receptor, signal transduction cascade, and cellular effects of stimulation through the EGFR. The ligand binding site serves as the receptor for ligands such as EGF and TGF-α. Many blocking anti-EGFR monoclonal antibodies prevent ligand binding and subsequent receptor signaling. Small-molecule tyrosine kinase inhibitors compete with the protein substrate or ATP for binding to the tyrosine kinase domain, preventing both autophosphorylation of the receptor and phosphorylation of target proteins that function in the signal transduction cascade. Radiation and certain chemotherapeutic agents can affect this pathway and in some cases may potentiate the effects of EGFR inhibitors.
[Reproduced with permission from Harari PM, Huang S-M. Modulation of molecular targets to enhance radiation. Clin Cancer Res 6:323-325, 2000]

14%-91%),[3,4,18] head and neck (greater than 80%),[5,6] lung up to 55%),[19-22] and ovarian cancers (35%-70% of primary tumors).[4,10,23] In some studies, EGFR overexpression was found to serve as a prognostic marker, suggesting a role for this receptor tyrosine kinase in cancer progression. Second, induction of EGFR overexpression in vitro leads to a transformed phenotype in avian fibroblasts.[24] The *v-erbB* oncogene is a mutant, truncated consitutively activated form of EGFR, which leads to the development of erythroleukemia and fibrosarcoma in chickens.[25,26] Finally, in a variety of models, abrogation of EGFR-mediated signal transduction through the use of antibodies or other tyrosine kinase inhibitors (as described in detail in this monograph) results in suppression of tumor cell growth and increased survival in tumor-bearing animals. These data support the idea that ligand binding to EGFR and subsequent tyrosine kinase activation may play a role in tumor progression.[27] Consequently, inhibition of EGFR expression and/or function could inhibit one or more key events in the growth and progression of tumors.

In order for tumors to metastasize, tumor cells must be capable of several essential processes. These include stromal and vascular invasion, embolization, survival in the circulation, arrest in a distant capillary bed, and extravasation into and multiplication in organ parenchyma.[28] The EGFR has been implicated in several pathways that affect tumor cell survival and apoptosis, angiogenesis, motility, and invasiveness (Figure 1). Therefore, inhibition of EGFR activity potentially could affect multiple aspects of tumor growth, progression, and metastasis.

Cell Survival and Apoptosis

Data suggest that the EGFR pathway may regulate cell survival since many EGFR-positive tumors depend on EGF or TGF-α stimulation (via autocrine or paracrine mechanisms) and subsequent EGFR-activated signal transduction for survival. In some cell lines EGF has been demonstrated to prevent apoptosis (programmed cell death) or promote survival in cells that overexpress EGFR.[29] EGF stimulation of breast adenocarcinoma cells also protected against apoptosis triggered by Fas, a cell death receptor induced by chemotherapeutic drugs.[30]

Conversely, inhibition of EGFR stimulation was found in many studies to block cell cycle progression and lead to apoptosis. Anti-EGFR antibodies were shown to promote apoptosis in a variety of tumor cell lines,[29,31-34] and similar effects were found using EGFR tyrosine kinase inhibitors[31,34,35] and antisense EGFR oligonucleotides.[36] Wu and colleagues found that the anti-EGFR monoclonal antibody 225 induced G_1 cell cycle arrest and apoptosis in the DiFi human colorectal carcinoma cell line.[32] Other tyrosine kinase inhibitors were also shown to induce apoptosis or DNA fragmentation (an indicator of apoptosis) in a variety of human tumor cells in vitro or in xenograft models.[34-39] These data suggest that activation of growth factor receptors such as EGFR may have a role in promoting cell survival in some tumors, and that inhibition of these pathways may promote programmed cell death in addition to growth inhibition or effects on angiogenesis or metastasis.

Angiogenesis

Angiogenesis is known to be critical for tumor growth, survival, and metastasis. Growth factors and their receptors, including EGF and EGFR, are thought to play a role in tumor angiogenesis as evidenced by numerous studies in the literature.[40] Coexpression of TGF-α and EGFR is highly correlated with microvessel density in invasive breast cancer.[41] TGF-α was shown to promote expression of vascular endothelial growth factor (VEGF), a well-characterized angiogenic growth factor that induces the growth and permeability of blood vessels.[42,43] While both EGF and TGF-α bind to EGFR, TGF-α was found to be more angiogenic in the hamster cheek pouch bioassay.[15] These preclinical observations are supported by clinical studies examining angiogenesis and EGFR expression in tumors. For example, in patients with stage I-III non-small cell lung cancer (NSCLC), univariate analysis demonstrated that sex, nodal status, stage, tumor size, microvessel count, and overexpression of the EGFR ligand amphiregulin significantly correlated with overall survival, with a lower probability of

survival in patients whose tumors expressed high levels of amphiregulin and high microvessel count.[22]

Conversely, blocking EGF binding can inhibit angiogenesis or the production of angiogenic factors. Treatment of EGFR-positive A431 human epidermoid carcinoma cells with anti-EGFR IMC-C225 antibody caused a dose-dependent inhibition of VEGF production in vitro.[44] This effect also occurred in vivo, and was accompanied by decreased tumor angiogenesis in established A431 tumors. IMC-C225 was found to inhibit in vitro production of VEGF, interleukin-8, and basic fibroblast growth factor (bFGF) in a highly metastatic human transitional carcinoma cell line.[45] When administered to nude mice with established orthotopic tumors, this antibody caused significant tumor regression and inhibition of metastasis, along with a reduction in neovascularization; treatment with the combination of this antibody and paclitaxel resulted in greater inhibition of tumor growth and metastasis compared to either agent alone.[46] The tyrosine kinase inhibitor genistein and the receptor tyrosine kinase inhibitor PD166285 were reported to inhibit angiogenesis in mice with mammary tumors, and genistein also decreased microvessel formation in bladder tumors.[47,48] These results suggest that disruption of EGF-dependent pathways by EGFR inhibitors is mediated in part through subsequent downregulation of angiogenic factors such as VEGF and bFGF. This may result in a partial suppression of angiogenesis, which could add to the therapeutic value of these inhibitors if this effect can be confirmed in clinical trials.

Cell Motility and Metastasis

Several studies have demonstrated that the EGF pathway is involved in regulating tumor cell motility and metastasis.[49-52] For example, EGF was shown to stimulate motility and metastasis in HER2-overexpressing SKBR3 breast cancer cells.[53] Similarly, human breast cancer cells transfected to overexpress EGFR could be induced to migrate through an artifical membrane in vitro by treatment with EGF.[54] Treatment of EGFR-transfected human glioma cells with TGF-α stimulated cell motility,[55] while EGF increased the invasiveness of glioma cells in vitro.[50] EGF also enhanced the random motility of human squamous cell carcinoma cell lines in a concentration-dependent manner.[51]

Some of these effects may be mediated through modulation of matrix metalloproteinases (MMPs).[56] This is supported by the threefold increased invasiveness seen in bladder transitional carcinoma cells (TCC) following stimulation with EGF in vitro, which could be inhibited by the anti-EGFR monoclonal antibody (MAb) IMC-C225; these results correlated with MMP-9 activity.[57] Furthermore, IMC-C225 treatment of nude mice bearing orthotopic TCC tumors resulted in inhibition of tumor growth and metastasis and decreased MMP-9 expression. Genistein, but not PD153035, downregulated expression of MMP-9 in cultured tumor cells,[56,58] although PD153035 was found to inhibit EGF-stimulated adhesion of tumor cells to extracellular matrix proteins in vitro.[59] MMP-1 was induced by EGF in two human bladder cancer cell lines in vitro, and was also found in the urine of patients with bladder carcinoma.[60] Whether MMPs have a causal role in EGFR-mediated tumor cell adhesion and invasiveness remains to be determined.

EGFR was shown to regulate cell-cell adhesion in the MDA-MB-468 breast carcinoma cell line, since activation with EGF inhibited cellular aggregation and led to the dissociation of the cell adhesion molecule E-cadherin and the actin cytoskeleton.[61] Using a different assay system, tumor cell adhesions induced by EGF could be inhibited using an anti-EGFR ligand-blocking antibody or a potent tyrosine kinase inhibitor.[59] Signaling from the receptor tyrosine kinase family members has been linked to temporal and spatial changes of the actin-based cytoskeleton through the actin-associated protein gelsolin.[62] The HER2/EGFR heterodimer activates phosphoinositides (particularly PIP2, phosphoinositol 4,5-diphosphate) via phospholipase C-gamma,[63] which in turn activates gelsolin. Furthermore, heregulin stimulation has been shown to promote physical attractions between p21-activated kinase 1, actin, and HER2, promoting cellular invasion via actin cytoskeletal

modifications.[64] Further research is needed to ascertain the clinical relevance of these EGFR-related changes in tumor cell adhesion, motility, and metastasis.

Detection of Epidermal Growth Factor Receptor Expression

Several different methods have been used to detect EGFR expression in tumor tissues. These include immunohistochemistry (IHC) for detection of protein expression,[6,65] fluorescence *in situ* hybridization (FISH) for detection of gene amplification,[66,67] competitive radioligand binding assay,[20,65] solid matrix blotting techniques (such as Southern and Northern blots),[68-70] reverse transcriptase-polymerase chain reaction (RT-PCR),[71] and ELISA.[72,73] Both IHC and FISH have advantages and disadvantages in terms of sensitivity, specificity, ease of use, specialized equipment required, and technical expertise needed (Table 1). The majority of published studies examining EGFR expression in tissues have relied on IHC due in part to its relative simplicity, speed, use of commonly available equipment, and minimal need of reader expertise.

Antibody-based testing methods including IHC are associated with a number of technical problems that potentially can affect the reliability and interpretation of the data from these studies, as has been described for HER2 detection.[74] Variation in specificity, sensitivity, and purity of anti-EGFR antibodies used for immunohistochemical detection may produce differences in staining, intensity, and the percent of positively stained cells. Some EGFR antibodies with lower specificity have been shown to cross-react with other related antigens, such as HER2. Other factors that may affect the detection of EGFR by IHC include variability in the detection of bound primary antibody (e.g., using FITC- or immunoperoxidase-labeled secondary antibodies or amplification steps) and in fixation or tissue pretreatment procedures (antigen retrieval). The lack of standardized scoring criteria to define a cutoff signal level that defines overexpression and the percent of stained cells required for a section to be scored as positive also contribute to this variability. Because EGFR is expressed in many normal epithelial tissues, there is a continuum of expression levels, and no clear agreement exists in the literature as to what constitutes high expression, overexpression, or normal expression. Additionally, inter-reader scoring differences may also contribute to varying results. Use of a standardized IHC system with a highly specific antibody and a standardized scoring system may reduce the variability often associated with this method. Comparative staining of fixed, embedded cell lines expressing a range of EGFR levels may also be useful to optimize and add quality control to IHC assays.

Table 1. Issues Related to Detection of EGFR Expression in Tumors

Immunohistochemistry (IHC)
- Variation in anti-EGFR antibodies and other reagents (lack of standardized methods)
- Frequent nonuniform staining
- Fails to detect EGFR gene amplification if present without protein overexpression
- Need for common grading criteria and scoring system
- Possible loss of signal over time or due to fixation methods
- Requires special antibodies to detect EGFR variants

Fluorescence *In Situ* Hybridization (FISH)
- EGFR gene not always amplified in tumors
- Higher cost
- Limited availability
- Need for specialized equipment/training
- Requires special probes to detect EGFR variants

Other detection methods have more significant limitations. FISH has been employed to detect amplification of EGFR and other genes in tumors, such as HER2 amplification in breast cancer.[74] However, while some EGFR-overexpressing tumors exhibit EGFR gene amplification (e.g., approximately 40% of human glioblastomas),[8] many others have a low frequency of amplification.[75,76] Since FISH can not detect protein overexpression in the absence of gene amplification, its use to detect EGFR overexpression is limited.

The higher cost, need for specialized equipment, and reader expertise are also problematic for FISH-based assays. While HER2 shed extracellular domain has been detected in the serum of some patients with HER2-overexpressing breast cancer,[77] circulating EGFR receptor generally has not been found in patients whose tumors overexpress this protein. Consequently, detection of serum EGFR by ELISA can not be used routinely. RT-PCR has been used to detect increased EGFR mRNA in head and neck cancers that were undetectable using Northern blot analysis.[71] This and other newer methods, however, are more research-oriented and are generally not available for routine screening purposes in clinical laboratories.

Currently, no standardized or commercially available test for measurement of EGFR expression exists. Institution of a standardized IHC assay using defined antibodies, reagents, positive and negative controls, and accepted scoring criteria would significantly reduce much of the variability in the detection and analysis of EGFR-positive tumors and might facilitate comparison of results from different studies and laboratories. Use of a defined panel of anti-EGFR antibodies, including those capable of detecting known EGFR variants, could also aid in detecting and characterizing EGFR-positive tumors that might otherwise escape detection with a single primary antibody. Work is in progress to develop such a standardized IHC test for routine detection of EGFR expression in tumor tissues. Human tumor pretesting to document EGFR overexpression prior to anti-EGFR treatment may be necessary on tumor types with variable EGFR overexpression, such as colorectal or breast cancer.

Epidermal Growth Factor Receptor Variants

Numerous variants of the EGFR have been detected that are associated with certain tumor types. The most common variant is EGFRvIII, caused by deletion of exons 2 to 7.[78] EGFRvIII is not found in normal tissues but is expressed on the cell membrane in certain tumors including 50% of all gliomas, in pr state cancer, and in subsets of breast and non-small cell lung cancers.[73,79-81] This variant possesses a constitutively activated tyrosine kinase and can cause ligand-independent transformation of cell lines, although it is incapable of ligand binding and receptor dimerization.[79] Specific MAbs have been isolated that can detect this variant on tumor cells by immunohistochemical means, permitting the identification of EGFRvIII in certain tumors that may not bind antibodies against wild-type EGFR.[73,80] The tumor-specific expression of this variant, coupled with rapid internalization of EGFRvIII-MAb complexes, suggests that targeted therapy of anti-EGFRvIII using MAbs or immunoconjugates may be useful for gliomas expressing this variant.[81,82]

Epidermal Growth Factor Receptor Expression in Human Tumors

EGFR is expressed on normal cells at levels ranging from 20,000 to 200,000 receptors per cell.[83] However, receptor levels can be much higher in malignant cells. MDA-468 breast cancer cells, which have an amplified EGFR gene and overexpress the receptor protein, may have up to 2 million surface EGF receptors per cell.[2] High levels of EGFR expression have also been observed in human tumors in which the EGFR gene is not amplified, such as renal carcinomas.[70,84] This increased level of expression found in many tumor types provides the rationale for targeting the EGFR in cancer therapy.

Tumor cells in culture are usually less dependent on exogenous growth factors for proliferation and survival than normal cells. This is presumably due to autocrine stimulation of the receptor by production of EGF or TGF-α from the tumor cells, which completes the feedback loop by binding to its own receptors. In support of this hypothesis, coexpression of EGFR and TGF-α was observed in nearly 40% of lung tumors.[85] Studies of the MDA-468 breast cancer cell line have detected production of a TGF-α–like factor

that is secreted into the medium and can compete with EGF binding to EGFR.[2] Tumor cells therefore may not require increased expression of EGFR for continued survival, just a functional autocrine loop.

Overexpression of EGFR in tumor cells can be achieved through different means. Amplification of growth factor receptor genes such as EGFR is one mechanism by which tumor cells can increase the number of cell surface receptors. This results in an increased number of ligands binding to the EGFR, which can further stimulate cell proliferation. EGFR gene amplification has been observed in a number of tumor types, such as glioblastomas (approximately 40% of cases),[68] astrocytomas (45%),[86] and NSCLC (13%).[76] EGFR may also be overexpressed in the absence of gene amplification,[76,87,88] presumably through mutations that increase EGFR transcription, mRNA translation, or stability of the protein. Additionally, EGFR mutants have been identified in some gliomas and NSCLC that have a constitutively active tyrosine kinase,[73,78] suggesting a role for high-level EGFR activity rather than EGFR verexpression in subsets of these cancers.

Higher levels of EGFR expression have been reported in many solid tumors and cancer cell lines (reviewed by Salomon et al, 1995; Woodburn, 1999).[5,89] These solid tumors include head and neck, esophageal, gastric, colon, pancreatic, non-small cell lung, renal, bladder, breast, ovarian, cervical, prostate, papillary thyroid, and laryngeal cancers. Increased EGFR expression also has been reported in melanomas, glioblastomas, and meningiomas. In certain tumor types, EGFR expression is very common. EGFR expression has been detected in 80% to 100% of head and neck squamous cell carcinomas,[5,6] and in 40% of glioblastomas.[68,88] The levels of EGFR expression and activity vary widely with tumor type, due in part to differences in detection methods. The range of EGFR overexpression observed for a given tumor type among different studies, and for various

Table 2. EGFR Overexpression in Selected Human Tumors

Tumor Type	Percentage of Tumors Expressing EGFR (range)	Expression in Corresponding Normal Tissue	References
NSCLC	40-80%	+	Salomon (1995)[5]; Rusch (1997)[21]; Fontanini (1998)[22]; Fujino (1996)[20]
Renal Carcinoma	50-90%	+	Salomon (1995)[5]; Yoshida (1997)[90]
Breast	14-91%	+	Klijn (1992)[3]; Walker (1999)[91]; Beckman (1996)[16]; Bucci (1997)[4]
Ovarian	35-70%	31% (low-level expression)	Salomon (1995)[5]; Fisher-Colbrie (1997)[23]; Bartlett (1996)[10]
Glioma	40-50%	-	Salomon (1995)[5]; Rieske (1998)[7]; Ekstrand (1991)[88]
Pancreatic	30-50%	+	Salomon (1995)[5]; Uegaki (1997)[92]
Head and Neck	80-100%	+	Salomon (1995)[5]; Grandis (1996)[8]
Colon	25-77%	+	Salomon (1995)[5]; Messa (1998)[9]
Bladder	31-48%	+ (only in basal epithelium)	Salomon (1995)[5]; Chow (1998)[12]

types of tumors, is illustrated in Table 2. Despite the variable expression seen among studies with respect to percentages and absolute protein levels, there is substantial evidence for increased EGFR expression in multiple tumor types.

Data from some studies suggest that in animal models EGFR expression may be greater in tumor metastases compared to the primary tumor. Increased EGFR expression was found in metastases, but not in the primary tumors, in nude mice xenografts of A431 epidermoid carcinoma.[93] In tumor cells isolated from human colon carcinoma biopsies, highly metastatic cells had EGFR mRNA levels that were more than fivefold greater than low metastatic cells.[69]

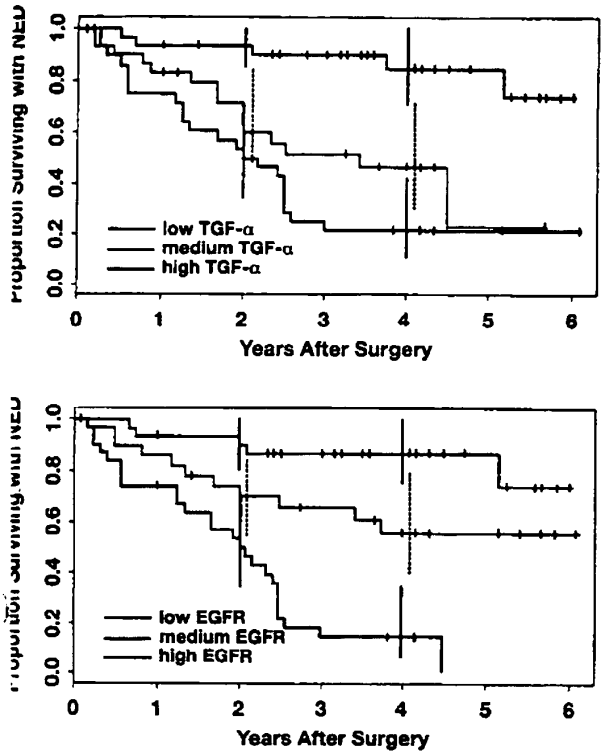
Epidermal Growth Factor Receptor Expression as a Prognostic Factor

In light of the frequent overexpression of EGFR in tumors and preclinical data supporting the role of EGFR in tumor progression, numerous studies have been conducted to determine if EGFR expression can serve as a prognostic factor in patents with cancer.[3,10-14,19,23,88,96,100,101] There is considerable debate in the literature over the value of EGFR as a prognostic indicator. In some studies EGFR expression has been associated with increased risk of recurrence, reduced survival, advanced tumor stage, and increased risk of metastasis (Table 3).[10,13,14,23] For example, in patients with head and neck cancer, the EGFR level may be useful for predicting tumor recurrence and appears to be superior to other prognostic indicators for this disease. Grandis et al found that in

Table 3. Prognostic Significance of EGFR in Selected Human Cancers

Tumor Type	Observations	References
NSCLC	EGFR expression correlated with shorter overall survival	Volm (1998)[13]
	Correlated with poor prognosis	Veale (1993)[97]
	Correlated with high metastatic rate	Pavelic (1993)[94]
Breast	EGFR expression correlated with worse overall survival and relapse-free survival when follow-up is short (1-4 years); correlation not significant at 10-year follow-up	Klijn (1992)[3]; Klijn (1994)[96]
	EGFR negatively correlated with ER/PR status	Klijn (1992)[3]
Ovarian	EGFR expression associated with poor prognosis	Bartlett (1996)[10]
	EGFR expression associated with poor overall and disease-free survival	Fischer-Colbrie (1997)[23]
	EGFR expression associated with resistance to chemotherapy	Niikura (1997)[95]; Fisher-Colbrie (1997)[23]
Glioma	EGFR expression levels correlated with degree of differentiation	Ekstrand (1991)[88]
Pancreatic	Coexpression of EGFR with EGF/TGF-α associated with advanced stage, tumor size, and decreased survival	Yamanaka (1993)[101]
Head and Neck	EGFR significant predictor of disease-free survival	Grandis (1998)[11]
	Correlated with overall survival in laryngeal carcinoma	Maurizi (1996)[14]
Colon	Patients with higher percentage of EGFR-expressing cells have poorer prognosis	Mayer (1993)[98]
Bladder	EGFR expression associated with increased risk of recurrence	Chow (1997)[12]; Turkeri (1998)[99]
	EGFR expression higher in invasive tumors	Neal (1989)[100]

Figure 2. Disease-Free Survival as a Function of TGF-α and EGFR Expression in Patients With Head and Neck Squamous Cell Carcinoma



Kaplan-Meier disease-free survival curves for 91 patients with head and neck squamous cell carcinoma. Levels of TGF-α and EGFR were measured by immunohistochemistry and quantified by computerized image analysis, then classified as low, medium, and high expressors. Vertical lines denote 95% confidence intervals at 2 and 4 years following surgery. In this study, the levels of EFGR protein (P=.0001) or TGF-α protein (P=.0001) were the strongest predictors of decreased disease-free survival.

Abbreviation: NED, no evidence of disease.

Reproduced with permission from Grandis JR, Melhem MF, Gooding WE, et al. Levels of TGF-α and EGFR protein in head and neck squamous cell carcinoma and patient survival. J Natl Cancer Inst 90:824-832, 1998. Reproduced by permission of Oxford University Press.]

addition to EGFR, TGF-α tumor expression was also a prognostic factor in head and neck cancer, and the expression level of both markers correlated with disease-free survival (Figure 2).[6,11] Expression of EGFR, TGF-α, and EGF is also correlated with risk of recurrence in bladder cancer.[12,99] A significant correlation between EGFR expression in tumors and a shorter time to disease recurrence and/or survival was observed in ovarian, laryngeal, squamous cell lung, and node-positive breast cancer.[13,14,102] In ovarian cancer, patients with high EGFR expression were more likely to have tumor recurrence.[23] In NSCLC, EGFR expression correlated with high metastatic rate or tumor invasiveness[19] and a trend toward worse overall survival,[85] and in bladder cancer and melanoma EGFR expression correlated with metastasis.[100,101]

Conflicting data exist even within studies of a given tumor type. In colorectal cancer, 12 studies evaluating EGFR expression found no correlation between receptor expression and histologic stage, tumor grade, proliferative index, or survival.[5] Subsequent studies, however, demonstrated high EGFR expression correlated with metastatic potential in this disease.[69] In an early report, EGFR expression was associated with a better outcome for patients with ovarian cancer,[104] whereas more recent studies indicate that EGFR positivity correlates with poor outcome.[23,105] Some of these prognostic studies were likely underpowered due to insufficient numbers of patients, making statistically significant conclusions unreliable, or may have utilized non-validated methods.

A large meta-analysis conducted by Klijn et al identifies some additional problems associated with studies in which the prognostic significance of EGFR was evaluated.[3,96] The study analyzed results of 40 different groups of investigators who examined EGFR expression in patients with primary breast cancer. EGFR expression correlated with tumor grade in 10 of 18 studies, tumor size in 2 of 17 studies, nodal status in 5 to 9 of 20 studies, and proliferative index in 3 of 9 studies. Associations between relapse-free survival and EGFR expression were dependent on time of follow-up, with more significant correlations found for short-term follow-up (1-4 years) than for long-term follow-up (>10 years). However, when the entire population of 5323 patients enrolled in all 40 studies was analyzed, significant correlations were found for EGFR expression and nodal status, tumor ploidy, and proliferative index. The

results were quite variable when data were analyzed for each study individually, however, because patient numbers were small and the studies were therefore weak in statistical power. Caution therefore should be exercised when evaluating prognostic data for other tumors, in addition to breast cancer. Larger-scale studies are warranted to strengthen correlations between EGFR and prognosis, metastatic potential, or survival for specific tumor types. Such studies may also reveal whether the use of EGFR expression in combination with other variables may provide greater prognostic value.

Epidermal Growth Factor Receptor as a Predictor of Response to Therapy

Chemotherapy

Alterations in chemosensitivity have been noted in preclinical studies of EGFR-overexpressing tumor cell lines. Indeed, higher levels of expression of drug-resistance–related proteins (topoisomerase II, P-glycoprotein) are found in untreated patients with EGFR-positive renal tumors.[106] Ogawa et al measured EGFR expression and cisplatin sensitivity in tumor tissues from 84 patients with lung cancer. EGFR expression was significantly higher in tumors that were resistant to cisplatin compared to cisplatin-sensitive tumors.[107] Similarly, patients with ovarian cancer who have EGFR-positive tumors or increased TGF-α expression have a lower rate of response to chemotherapy with cisplatin compounds compared to patients with lower EGFR levels.[23] Santini et al reported that patients with head and neck tumors in which EGFR expression levels were >100 fmol/mg protein had a lower probability of response to chemotherapy than did patients with EGFR levels <100 fmol/mg protein.[108] In a study of 82 patients with head and neck cancer treated with cisplatin/5-fluorouracil ± folinic acid as induction or concomitant therapy, Etienne and colleagues also found lower response rates among patients with higher levels of EGFR.[109] Together, these data suggest that higher levels of EGFR may be associated with chemoresistance in some tumors. However, presently there are insufficient data to suggest using EGFR expression as a predictor for response to chemotherapy. It is probably more valuable to consider modifying EGFR activity to enhance chemotherapy.

Radiotherapy

An association between EGFR expression and clinical radioresistance has been reported in patients with cancer. Maruzi and colleagues reported a correlation between EGFR overexpression and response to radiotherapy in human head and neck cancers.[14] EGFR expression was a significant and independent prognostic indicator for overall survival and recurrence-free survival after radiation therapy in patients with astrocytic gliomas.[110] Recently, Pillai et al noted that patients who had residual or recurrent disease after radiotherapy for cervical cancer had more EGFR expression than those patients who were disease-free.[111] While preclinical studies indicate that EGFR inhibition can sensitize many tumor cells to ionizing radiation, in vitro sensitization with cell lines may not reflect the prognostic implications of EGFR overexpression in vivo. Further study is therefore needed to evaluate the relationship of EGFR expression and enhanced radiosensitization in vivo.

Inhibition of the Epidermal Growth Factor Receptor

Over the past several years, it has been recognized that antibodies and small-molecule inhibitors can be used therapeutically to perturb signaling at the cellular level and promote cell death. Since the EGFR is an integral component of the EGF signaling pathway involved in regulating tumor growth, a logical approach to cancer therapy is to try t block the function of this receptor and thus inhibit cell proliferation and tumor progression. A variety of approaches have been developed to target the EGFR for antitumor therapy. These agents, discussed in detail below, include monoclonal antibodies directed against the receptor, which block binding of ligand (EGF or TGF-α) and receptor-activated cell growth; synthetic tyrosine kinase inhibitors

Table 4. Approaches to Inhibition of EGFR

Anti-EGFR Antibodies
- Antibody binds to EGFR on cell surface
- Ligand binding to receptor blocked
- Signal transduction cascade blocked
- Receptor-antibody complex internalized

Tyrosine Kinase Inhibitors
- Inhibitors bind intracellularly to EGFR tyrosine kinase
- Kinase activity inhibited
- Signal transduction cascade blocked

Ligand Conjugates
- EGFR ligand (e.g., EGF, TGF-α) conjugated to toxin (ricin, *Pseudomonas* exotoxin)

Immunoconjugates
- Anti-EGFR antibody conjugated to ricin

Antisense Oligonucleotides
- EGFR or TGF-α antisense oligonucleotides bind to DNA or RNA
- May be delivered using liposome technology

that act directly on the cytoplasmic domain of the EFGR, preventing signal transduction and cell proliferation; and ligand conjugates, which bind specifically to the EGFR and deliver a toxic, lethal payload following ligand-toxin internalization (Table 4).

EGFR Inhibition by Anti-EGFR Antibodies

A number of antibodies directed against the EGFR have been generated by various groups. These were shown to block ligand binding to the receptor and to inhibit EGF-stimulated tyrosine kinase activity and tumor growth.[32,73,112-119] Following antibody binding, the EGFR-antibody complex is internalized and degraded, preventing receptor reutilization by the cell.[17,82]

EGF receptors from various human epidermoid carcinoma cells were used to immunize mice to raise MAbs to EGFR. Two such antibodies, M225 and M528, were found to compete with EGF binding to EGFR, inhibit EGF-induced tyrosine kinase-dependent phosphorylation, and downregulate EGFR expression by inducing receptor internalization. These antib dies inhibited EGF-induced anchorage-dependent and anchorage-independent growth of EGFR-positive cells in vitro as well as TGF-α–induced growth of A431 epidermoid carcinoma cells.[119] Treatment of the MDA-468 breast cancer line (which has an amplified EGFR gene and overexpresses this receptor[2]) with M225 and other anti-EGFR MAbs inhibited growth, presumably by blocking the signal transduction needed for cell survival.

Early clinical trials using murine MAbs demonstrated that many patients developed a human anti-mouse antibody (HAMA) immune response against these foreign proteins, resulting in accelerated clearance and limited therapeutic potential. To overcome the HAMA response, genetic engineering has allowed for the production of chimeric (or partially "humanized") MAbs. Such antibodies retain only a small portion of murine protein sequences that are responsible for antigen binding, with the remainder of the molecule composed of human immunoglobulin. M225 therefore was used to create a chimeric version, IMC-C225, which is more effective than M225 at inhibiting the growth of established tumor xenografts and has a binding affinity ($1\text{-}2 \times 10^{-10}$ M) that is approximately 1 log greater than that of the natural EGFR ligand ($1\text{-}2 \times 10^{-9}$ M).[120] This binding affinity also approximates the theoretical ideal antibody affinity proposed by Weinstein et al, since binding affinities greater than this proposed value could result in reduced tumor distribution due to a binding-site barrier.[121]

Although IMC-C225 induces EGFR receptor dimerization similar to that seen with endogenous ligands, it can block the EGF-induced activation, autophosphorylation, and the internalization of the receptor.[17] Several studies have demonstrated the ability of this antibody to inhibit the growth of EGFR-positive tumor cells in vitro and in vivo. For example, treatment with IMC-C225 resulted in dose-dependent growth inhibition of EGFR-positive SK-RC-29 renal cell carcinoma xenografts, with decreased tumor volume and increased survival.[122] IMC-C225 and other similar MAbs also inhibited the growth of multiple EGFR-positive tumors, such as pancreatic carcinoma,[123] breast carci-

noma,[34] colorectal carcinoma,[32] prostate cancer,[17,124] and A431 epidermoid carcinoma xenografts,[116,120] with the induction of apoptosis demonstrated in some studies.[17,32]

Since EGFR is overexpressed in nearly all head and neck squamous cell carcinomas, one important question is whether anti-EGFR MAbs can be used therapeutically to target these tumors. Cell lines derived from primary, recurrent, and metastatic head and neck tumors were used to demonstrate growth inhibition with several such MAbs.[73,125,126] The combination of cisplatin with either ICR63 or ICR80 MAb produced enhanced growth inhibition in these cell lines.[72] In another study, treatment with these anti-EGFR MAbs decreased tumor cell growth in culture, and induced terminal differentiation and apoptosis.[125] Anti-EGFR MAb IMC-C225 could also radiosensitize EGFR-overexpressing head and neck cancer cell lines, increasing the degree of growth inhibition and induction of apoptosis.[126] These results suggest that anti-EGFR MAb may be useful in the treatment of head and neck cancers in combination with chemotherapeutic agents such as cisplatin or radiotherapy (see "Combination Therapy With Epidermal Growth Factor Receptor Inhibitors Plus Chemotherapy and Radiotherapy").

A novel approach to the use of anti-EGFR antibodies involves their combination with other MAbs targeting different tumor antigens, such as HER2. These receptors are known to heterodimerize upon binding of EGFR ligands, resulting in a high-affinity receptor.[127] Furthermore, overexpression of both EGFR and HER2 has been implicated in the pathogenesis of breast cancer. Transgenic mice that express high levels of a constitutively activated *neu* (HER2) gene develop mammary tumors.[128] Similarly, transgenic mice that express TGF-α in mammary epithelium develop mammary epithelial hyperplasia that progresses to focal mammary tumors following a long latency period, as do wild-type *neu* transgenic mice.[129-131] These two tyrosine kinases therefore may collaborate in the progression of some breast cancers. This hypothesis is supported by studies demonstrating coexpression of EGFR and HER2 in various human tumors (including breast cancer), which was correlated with stage of disease and indicated a worse prognosis compared with tumors that expressed either marker alone.[66,76,88,133] EGFR and HER2 coexpression also were identified in a majority of breast cancers with activation of HER2.[134]

Studies have examined the effects of combinations of anti-EGFR and anti-HER2 MAbs on tumor cells. Growth inhibition of OVCA420 human ovarian cancer cells was observed following exposure to either IMC-C225 or the anti-HER2 MAb 4D5; the combination resulted in enhanced inhibition, G_1 accumulation, and increased levels of the cyclin-dependent kinase (CDK) inhibitor $p27^{KIP1}$.[135] These data suggest that the combination of an anti-HER2 MAb (such as trastuzumab; Herceptin®) and an EGFR inhibitor (such as IMC-C225) may provide greater efficacy than either agent used alone in patients whose tumors are EGFR-positive and overexpress HER2.

Tyrosine Kinase Inhibitors

Another approach to blocking EGFR activity has involved the use of compounds that inhibit the tyrosine kinase activity of the receptor. Inhibition of tyrosine kinase activity prevents receptor autophosphorylation and the phosphorylation of proteins involved in the various EGFR signaling pathways. Theoretically, such an approach could inhibit EGF- or TGF-α–stimulated signaling and also signaling that is independent of growth factors. For example, deletion mutants that are constitutively active in the absence of EGF or TGF-α have been reported and are linked to some cancers (see "Epidermal Growth Factor Receptor Variants").[74,79-82] It should be noted, however, that MAbs specific for such deletion mutants exist,[82,83] which may also be capable of inhibiting these variant receptors. Lefkowitz and others also have recently reported that the EGFR tyrosine kinase can be activated by signaling from an adrenergic receptor.[136,137]

For tyrosine kinase inhibitors to be used therapeutically, they must be highly specific. The inhibitors studied to date

have varying levels of specificity. Several quinazoline derivatives have been developed that are more selective for EGFR than for other tyrosine kinase receptors. Many of these small-molecule inhibitors have significant antitumor activity, inhibiting both the proliferation of EGFR-overexpressing cells in culture and the growth of EGFR-positive tumor xenografts in mice. The growth-inhibitory characteristics of some of the more potent and selective tyrosine kinase inhibitors are reviewed below and are summarized in Table 5.

ZD1839 is an anilinoquinazoline with an IC_{50} of 20 nM for the EGFR tyrosine kinase. It inhibits EGFR autophosphorylation in cultured cell lines and the EGF-stimulated growth of oral carcinoma cell lines. ZD1839 has significant antitumor activity in a variety of human tumor xenograft models, including A549 lung, LoVo colon, and DU145 prostate tumors.[40,89,138,139] At a dose of 10 mg/kg, ZD1839 reduced growth rates of A431 human epidermoid carcinomas implanted in mice by 50%, and at a higher dose (200 mg/kg) caused regression of 1.5-g tumors to an undetectable size.[138]

CP-358,774 is an quinazoline analog with a nanomolar IC_{50} for inhibition of EGFR activity and high specificity for the receptor.[89] It inhibits proliferation of DiFi colorectal cancer cells and leads to a cell cycle arrest in both DiFi cells and human head and neck tumor cells.[35] This inhibitor has been shown to reduce tumor growth rates by 50% in mice implanted with human HN5 xenografts; however, at doses as high as 200 mg/kg, the inhibitor failed to inhibit proliferation of A431 xenografts.

PD153035, another tyrosine kinase inhibitor in the quinazoline family, has been tested extensively in preclinical studies.[36,60,125] This compound was found to inhibit proliferation of EGFR-overexpressing cells at micromolar concentrations.[140] PD153035 inhibits autophosphorylation of HER2 as well, but at much higher concentrations.

Table 5. Antitumor Activity of Selected Tyrosine Kinase Inhibitors

Tyrosine Kinase Inhibitor	Chemical Class	Inhibition of EGF-Stimulated Growth Proliferation	Antitumor Activity in Vivo
PD158780	Pyridopyrimidine	Nanomolar inhibition of EGF-stimulated thymidine incorporation in Swiss 3T3 cells	Inhibits tumor growth rate of A431 xenografts in mice
CP-358,774	Quinazoline	IC_{50}= 70 nM in contact-inhibited Fischer rat embryo cells*	Inhibits autophosphorylation in HN5 xenografts in mice
ZD1839	Quinazoline	IC_{50}=80 nM in KB oral carcinoma cells	Antitumor activity in mouse xenograft models including vulvar, NSCLC, prostate, ovarian, and colorectal cancers
CGP 59326A	Pyrrolopyrimidine	IC_{50}=1.38 μM in BALB mouse keratinocyte cell line†	Inhibited growth of EGFR-positive xenografts in mice, with little activity against xenografts expressing low levels of EGFR
PD153035	Quinazoline	IC_{50}=200 nM in human colon cancer cell lines‡	Inhibited growth of EGFR-positive head and neck and colon cancer cell lines

* Measured by monitoring BrdUrd incorporation (DNA synthesis).
† Monitored by methylene blue staining.
‡ Measured by MTS cell viability assay.

PD158780 is a pyrimidine derivative that can inhibit EGF-dependent receptor autophosphorylation at nanomolar concentrations in vitro.[141] In soft agar assays, PD158780 reduced clone formation of fibroblasts transformed with the EGFR or HER2 gene, but not the *ras* oncogene.[138] PD158780 has been shown to inhibit the growth of human tumors implanted in nude mice. Growth inhibition has also been observed for EGFR-transfected fibroblast tumors in nude mice using a 15-day oral administration schedule of PD165557, which was developed as a more soluble analog of PD158780.[142]

CGP 59236A is a pyrrolopyrimidine compound with high selectivity for EGFR. The IC_{50} for EGF-stimulated growth in BALB mouse keratinocyte line is 1.38 μM.[143] This compound was found to inhibit proliferation of EGFR-positive cells derived from lung, breast and prostate cancers.[143] It has been shown to suppress growth rates in A431 xenografts and cause growth delays in NCI-M596 xenografts.[143]

Other Inhibitors

Apart from antibodies and tyrosine kinase inhibitors, other approaches have been used to target the EGFR in tumor cells. A gene encoding a single-chain antibody that specifically binds to EGFR has been constructed. Following intracellular expression of the gene in EGFR-transformed 3T3 fibroblasts, this antibody inhibited EGF-induced activation and anchorage-independent cell growth in vitro.[144] Antisense therapy using DNA or RNA oligonucleotides is designed to prevent the translation of specific mRNA transcripts into protein. Antisense oligonucleotides directed at oncogenes and/or growth factors have been used to inhibit the growth of several human cancer cell lines.[145,146] When EGFR antisense RNA sequences were injected into mice bearing EGFR-overexpressing head and neck tumors, EGFR expression and tumor growth were suppressed and the rate of apoptosis increased. Treatment with EGFR sense constructs had no effect.[37] Human KB tumor cells incubated with EGFR antisense oligonucleotides in folate-PEG-liposomes also reduced EGFR expression and cell proliferation.[147] Antisense oligonucleotides directed against mRNAs of TGF-α and the EGF-related peptides amphiregulin and cripto were found to inhibit the growth of GEO human colon carcinoma cells in vitro.[148,149] However, the role of antisense therapy in the treatment of cancer remains to be defined. The extent of antisense sequence uptake into tumor cells is not clear, and treatment parameters such as dosing and scheduling still need to be determined. The possible adverse effects of injecting large quantities of short-chain oligonucleotides into the bloodstream are also unknown at present. Further research with EGFR antisense constructs to inhibit gene expression is underway.

Immunoconjugates of toxins to anti-EGFR antibodies have been developed that show activity in preclinical studies. Conjugates of the anti-EGFR MAbs IMC-C225 and 528 with ricin A chain, a potent protein synthesis inhibitor, have been shown to preferentially inhibit the growth of EGFR-positive tumor cell lines in vitro.[150-152] Since an inverse relationship exists between EGFR receptor number and cytotoxicity of these conjugates, some degree of selectivity in targeting EGFR-overexpressing tumors may be possible.

Conjugates between toxins and EGFR ligands were designed with the aim of developing toxic agents specific for EGFR-overexpressing tumor cells while minimizing nonspecific toxicity. A conjugate composed of EGF and *Pseudomonas* endotoxin (PE) was shown to be toxic toward EGFR-expressing HeLa cells in vitro.[153,154] Cellular uptake of this conjugate and cytotoxicity could be enhanced by various agents including thioridazine and adenovirus. Similar EGFR-specific cytotoxicity was seen in a series of EGFR-positive human tumor cell lines using a genetically engineered TGF-α–PE hybrid toxin.[155] The soybean-derived protein genistein, a general tyrosine kinase inhibitor that alone inhibits breast cancer cell lines, was found to inhibit the EGFR tyrosine kinase when coupled to EGF. Treatment of mice bearing human breast cancer xenografts with this conjugate inhibited tumor growth,

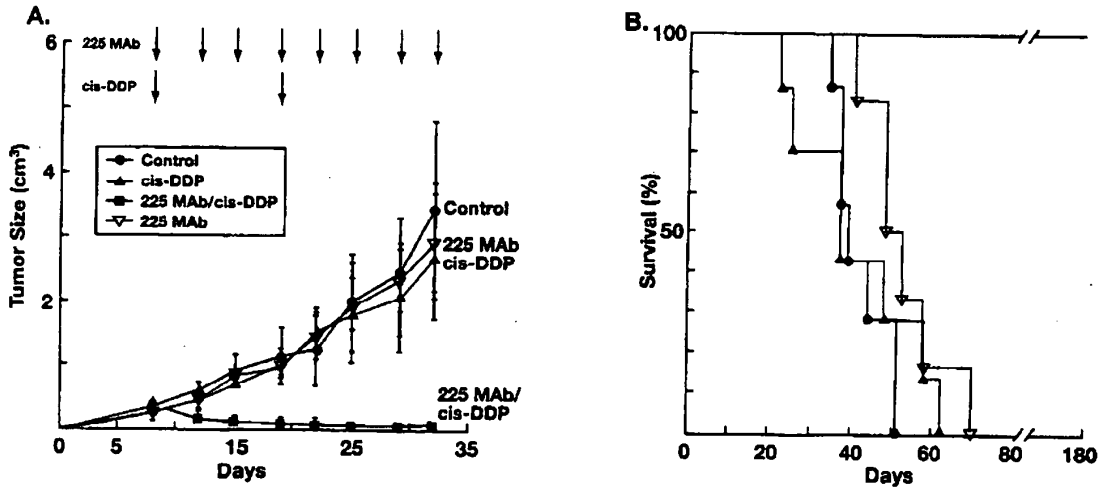
induced apoptosis, and improved survival.[39,59,156] In these studies the conjugate was found to be more potent compared to genistein alone. A ligand fusion toxin consisting of human EGF fused to the active moiety of diphtheria toxin ($DAB_{389}EGF$), which binds to and kills EGFR-positive cells through inhibition of protein synthesis, has been studied in a clinical trial.[157] Finally, an immunotoxin was created in which an anti-EGFR antibody (B4G7) was conjugated to gelonin, a ribosome-inactivating protein. This conjugate had significant cytostatic properties when administered to nude mice bearing EGFR-positive A431 tumors, but not against EGFR-negative tumors.[158] Further studies are warranted to ascertain the degree of in vivo toxicity and the clinical potential of these EGFR-targeting conjugates.

Combination Therapy With Epidermal Growth Factor Receptor Inhibitors Plus Chemotherapy or Radiotherapy

EGFR Inhibitors Plus Chemotherapy

Given the inhibition of cell growth and tumor proliferation observed using anti-EGFR antibodies alone, it was hypothesized that the combination of antibodies and standard antitumor therapy may have an enhanced or synergistic effect. Alterations in the expression or activity of EGFR (or its downstream molecular signals) might affect the sensitivity of tumor cells to chemotherapy or radiation therapy by potentiating the damage caused by these agents and/or inhibiting their repair.[159] This effect has been observed with other MAbs, where the combination of antibody and chemotherapy produces greater antitumor effect than either agent used alone. When the anti-HER2 MAb trastuzumab was combined with chemotherapeutic drugs such as paclitaxel, doxorubicin, cisplatin, and methotrex-

Figure 3. Inhibition of A431 Epidermoid Carcinoma Xenografts by Combination Treatment With Anti-EGFR Monoclonal Antibody C225 Plus Cisplatin



A431 cells (10^7) were implanted subcutaneously into nude mice and allowed to grow for 8 days. In A, the mice were given intraperitoneal injections of either phosphate-buffered saline (●); two injections of cisplatin (cis-DDP; 150 mg/25 g mouse weight) on day 8 and day 18 (▲); or 225 MAb, 1 mg/mouse, twice a week for 4 weeks; with (■) or without (▽) two injections of cis-DDP (150 mg/25 g mouse weight) on day 8 and day 18. Arrows indicate timing of drug and antibody treatment. The data are expressed as the mean tumor size ± SE (7 mice per group). In B, survival was determined over a 6-month period.

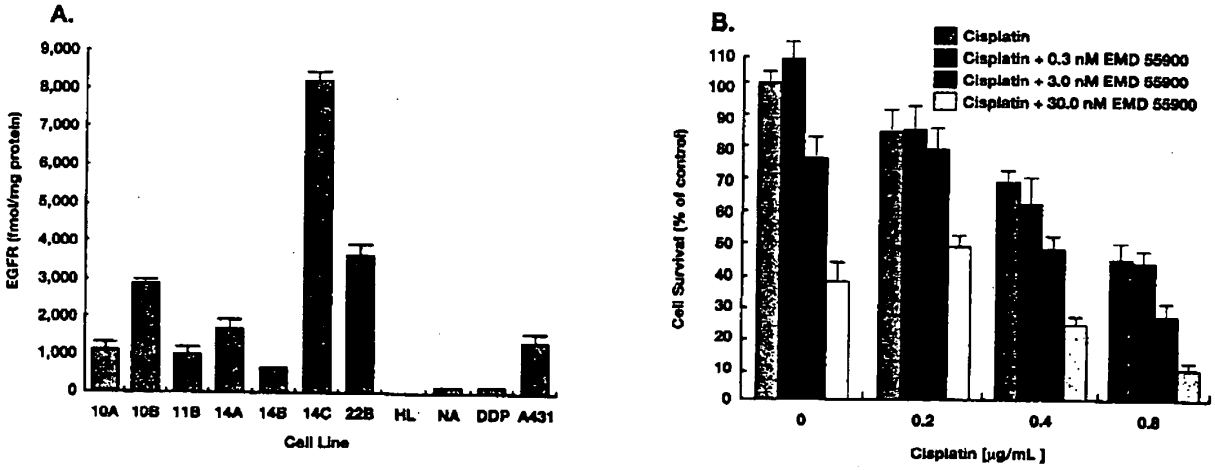
[Reproduced with permission from Fan Z, Baselga J, Masui H, et al. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus *cis*-diamminedichloroplatinum on well established A431 cell xenografts. Cancer Res 53:4637-4642, 1993]

ate, enhanced or synergistic growth inhibition of human breast and ovarian cancer xenografts was observed.[160-162] Similarly, the anti-CD20 MAb rituximab (Rituxan®) was found to act synergistically with doxorubicin in vitro, and could also revert chemoresistant human lymphoma cell lines to a chemosensitive state.[163]

In the case of the EGFR, enhanced cytotoxicity and inhibition of tumor growth have been observed in many studies using combinations of chemotherapy and various anti-EGFR antibodies. Concurrent treatment of KB carcinoma cells at the time of implantation with cisplatin plus the EGFR MAb 108 resulted in an enhanced antitumor effect.[117] Enhanced growth inhibition of human prostate cancer cells was seen using anti-EGFR MAb IMC-C225 combined with doxorubicin or cisplatin in vitro and in xenograft models.[17] The combination of IMC-C225 and cisplatin also caused regression in well-established A431 squamous cell carcinoma xenografts (Figure 3),[34] while IMC-C225 plus doxorubicin produced greater inhibition than either agent alone in MDA-468 xenografts.[116]

Exposure of topotecan-treated human cancer cell lines to IMC-C225 resulted in markedly enhanced growth inhibition and apoptosis, and this combination of agents caused near-complete tumor regression in established colon cancer xenografts.[164] Studies in MDA-MB-468 breast cancer cells have also shown potentiation of the antitumor activity of paclitaxel when given in combination with IMC-C225.[165] In EGFR-overexpressing human head and neck cancer cell lines, enhanced inhibition was seen with the anti-EGFR antibodies EMD 55900 and ICR-62 when combined with cisplatin (Figure 4).[73] Enhanced growth inhibition of breast cancer xenografts was also observed with the EGFR-specific protein kinase inhibitor CGP 59326A when combined with various chemotherapeutic agents (Figure 5).[143] Similarly, ZD1839 in combination with drugs such as cisplatin, carboplatin, paclitaxel, and etoposide markedly enhanced apoptosis in EGFR-positive GEO colon cancer cells and prolonged survival in mice bearing these xenografts[166]; combinations with cisplatin or paclitaxel have also demonstrated efficacy against LoVo colorectal xenografts.[139]

Figure 4. Growth Inhibition of EGFR-Positive Head and Neck Cancer Cell Lines by Cisplatin and Anti-EGFR Monoclonal Antibody EMD 55900



A) Differential EGFR protein expression in 10 squamous cell carcinoma head and neck lines. EGFR protein was measured by quantitative ELISA assay. B) In vitro inhibition of cell growth in the head and neck cell line UM-SCC 14C, which expresses high levels of EGFR, by cisplatin and the anti-EGFR monoclonal antibody EMD 55900. Cells were treated with 0, 0.2, 0.4, or 0.8 mg/mL cisplatin and 0, 0.3, 3.0, or 30.0 nM EMD 55900 for 72 hours, and percent of surviving cells was determined by colorimetric MTT assay. For both graphs, vertical bars represent standard error of the mean (n=3).

[Reproduced with permission from Hoffmann T, Hafner D, Ballo H, et al. Antitumor activity of anti-epidermal growth factor receptor monoclonal antibodies and cisplatin in ten human head and neck squamous cell carcinoma lines. Anticancer Res 17:4419-4426, 1997]

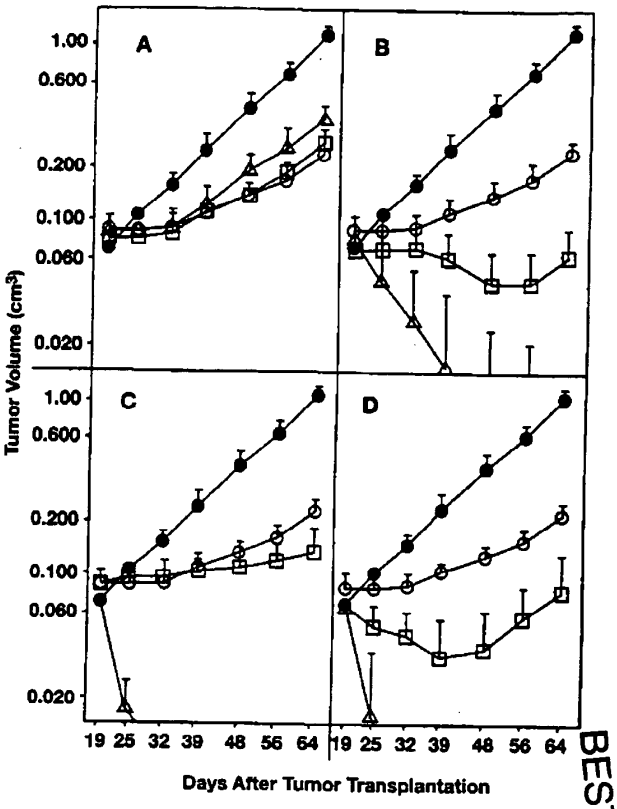
Mechanistically, the observed enhancement or synergy may reflect an imbalance between two signaling pathways: one favoring proliferation and survival, which is stimulated by ligand binding to EGFR and subsequent transduction of growth signals, and the other inducing apoptosis due to a cellular insult such as chemotherapy. DNA damage by chemotherapeutic agents normally results in cell cycle arrest in G_1 in order to repair the damage. If this cannot occur or is incomplete, apoptosis may occur. Therefore, growth factor restriction (e.g., blockage of ligand binding to EGFR) and treatment with DNA-damaging agents such as cisplatin may preferentially enhance apoptosis in tumor cells.[167] It should also be noted that in addition to EGFR overexpression, many tumors have other genetic alterations, such as mutations in the p53 tumor suppressor gene, that may alter the cellular response to chemotherapy or radiation therapy.[168]

EGFR Inhibitors Plus Irradiation

In addition to these interactions between EGFR and chemotherapy, many preclinical studies have demonstrated that inhibition of EGFR may sensitize tumor cells to ionizing radiation. Anti-EGFR MAb IMC-C225 was shown to radiosensitize head and neck cancer cell lines that overexpress the EGFR, inhibiting cell growth and enhancing the induction of radiation-induced apoptosis; these effects may be correlated with decreased EGFR autophosphorylation.[126,169,170] Similarly, EGFR tyrosine kinase inhibitors enhanced radiosensitivity in a breast cancer cell line.[171] Radiosensitization has also been demonstrated in vivo. Nude mice with established intracranial human gliomas that were treated with IMC-C225 MAb and concurrent irradiation had a significantly prolonged survival compared to mice treated with either therapy alone.[172] The radioresponse of A431 tumor xenografts (as measured by delay in tumor growth) was enhanced by a factor of three following treatment with three doses of C225 MAb.[173]

The basis for radiosensitization is undefined, but several mechanisms of action have been postulated. Radiosensitization by EGFR inhibitors may be due to alterations in cell cycle; inhibition of DNA repair resulting from radiation-induced DNA damage; or prevention of cell survival signals required during cell cycle arrest, leading to apoptosis.[174] (1) The enhanced cytotoxicity of radiotherapy plus anti-EGFR

Figure 5. Growth Inhibition of Human Breast Cancer Xenografts by EGFR-Specific Protein Tyrosine Kinase Inhibitor CGP 59326A and Chemotherapy



Human breast carcinoma MDA-MB486 xenografts were transplanted into female BALB/c nude mice. Treatment with CP 59326A and/or chemotherapy was initiated 19 days after transplantation and animals were monitored for tumor regrowth until day 64. Animals were treated as follows: (A) Placebo control (●) or CGP 59326A once daily p.o. for 44 consecutive days (○, 50 mg/kg; □, 10 mg/kg; △, 2 mg/kg). (B) CGP 59326A (○, 50 mg/kg p.o. once daily on days 19-63), doxorubicin (□, 9.0 mg/kg i.v. on days 19, 26, and 33), or a combination of doxorubicin and CGP 59326A (△). (C) CGP 59326A (○, 50 mg/kg p.o. once daily on days 19-63), paclitaxel (□, 20 mg/kg i.v. on days 19, 21, 23, 25, 27, 46, 48, 50, 52, and 54), or a combination of paclitaxel and CGP 59326A (△). (D) CGP 59326A (○, 50 mg/kg p.o. once daily on days 19-63), vinblastine (□, 1.5 mg/kg i.v. on days 19, 26, 33, and 40), or a combination of vinblastine and CGP 59326A (△).

[Reproduced with permission from Lydon NB, Mett H, Mueller M, et al. A potent protein-tyrosine kinase inhibitor which selectively blocks proliferation of epidermal growth factor receptor-expressing tumor cells in vitro and in vivo. Int J Cancer 76:154-163, 1998. © 1998 Wiley-Liss, Inc. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

MAb may be due in part to inhibition of DNA repair. Cells that cann t undergo cell cycle arrest t carry out DNA repair may undergo apoptosis. Inhibition with anti-EGFR antibodies reduced the level and activity of DNA-dependent protein kinase, an enzyme thought to have a role in repairing DNA double-strand breaks.[175] In the case of cisplatin, repair of a cisplatin-damaged plasmid vector was decreased in A431 cells treated with IMC-C225 MAb.[176] (2) Inhibition of EGFR tyrosine kinase activity by antibodies or kinase inhibitors prevents activation of downstream cell signals such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K). In many tumor lines inhibition of MAPK and PI3K results in increased radiosensitivity, enhanced DNA damage following irradiation, and/or apoptosis.[177-179] Recent data suggest that the pathway from EGFR to MAPK serves not only as a proliferation pathway but also in some instances as a cell survival pathway[180]; similar data highlights the importance of PI3K and Akt activity in cells that overexpress EGFR.[181] In tumor cells that depend on EGFR-mediated stimulation, inhibition of these pathways by a specific antibody or tyrosine kinase inhibitor may abrogate these cell survival signals, thus sensitizing cells to radiation and inducing apoptosis.

Clinical Experience in Targeting Growth Factor Receptors

In addition to the possible prognostic and predictive value of EGFR overexpression, there is growing evidence that EGFR expression may select a patient population that could benefit from anti-receptor–specific therapy. Chimeric and humanized antibodies have been used successfully in clinical trials for cancer therapy, including the anti-HER2 MAb, trastuzumab (Herceptin), a MAb directed against the receptor-like HER2 molecule, for treatment of HER2-positive metastatic breast cancer.[182] Trastuzumab typically does not result in HAMA despite repeated administration. Apart from the occurrence of infusion-related symptoms typically consisting of fever, chills, nausea, and fatigue (observed in about 40% of patients during the first infusion of trastuzumab, and with decreasing frequency thereafter), therapy with MAbs is generally safe. It should be noted, however, that toxicities can arise following MAb therapy (e.g., trastuzumab cardiotoxicity, particularly in combination with high-dose anthracyclines, or respiratory distress observed in some patients).

Phase I and phase II trials with selected EGFR inhibitors are in progress (reviewed by Woodburn).[89] These include the chimeric anti-EGFR MAb IMC-C225, the mouse anti-EGFR MAb 425 (EMD 55900), a fully humanized anti-EGFR MAb, and the tyrosine kinase inhibitors ZD1839 and CP-358,774.[183] A trial has also begun using a human recombinant EGF linked to a carrier protein, in an attempt to induce an immune response that would block ligand binding to the EGFR.[184] Clinical trials have been conducted with other anti-EGFR antibodies, including the fully human antibody ABX-EGF[185] as well as with the rat MAb ICR62.[114] The results of these trials in terms of safety and any indications of efficacy will be of great interest.

Summary

Activation of the EGFR receptor initiates signal transduction pathways that are necessary for cellular proliferation, survival, and tumor progression. Increased EGFR expression and activity have been documented in a number of human cancers. The significance of EGFR overexpression is highlighted by numerous studies that support EGFR as an indicator of poor prognosis for many cancers. The EGFR therefore represents a potentially important target for antitumor therapy. Two major approaches for inhibiting EGFR include the use of antibodies directed against the receptor to block ligand binding, and small molecules that inhibit the tyrosine kinase enzymatic function of the receptor. Both classes of molecules can inhibit EGF-stimulated proliferation of cultured cells, promote apoptosis, and inhibit pathways necessary for tumor survival and metastasis. Furthermore, both have been shown to be effective inhibitors of tumor growth in xenograft models.

Currently, the relative therapeutic value of anti-EGFR MAbs versus tyrosine kinase inhibitors is not known, but both classes of agents are currently in clinical trials. Because of the differences in mechanisms of action between MAbs and tyrosine kinase inhibitors in targeting distinct aspects of receptor function (i.e., ligand-receptor interaction versus tyrosine kinase activity), these two classes of agents may have disparate clinical effects and/or toxicities. Such differences in mechanism of action may facilitate the use of MAb and tyrosine kinase inhibitor combinations. Bos and colleagues found that the addition of IMC-C225 to the tyrosine kinase inhibitor PD153035 resulted in enhanced growth inhibition of A431 epidermoid carcinoma cells in vitro, despite the fact that concentrations of PD153035 were high to achieve complete inhibition of EGFR tyrosine kinase activity.[140] Thus, combined treatment strategies that target the EGFR and MAbs and a tyrosine kinase inhibitor may offer an advantage.

Early results from clinical trials with EGFR inhibitors suggest promise in the treatment of various cancers, with minimal toxicity noted to date. In light of the enhanced or synergistic inhibition of tumor growth and increased survival seen in preclinical studies with the combination of EGFR inhibitors and chemotherapeutics or radiation, anti-EGFR MAbs and tyrosine kinase inhibitors are likely to be used clinically in such combinations for maximal efficacy. While single-agent MAb therapy has been used successfully in some patients, combinations of antibodies and chemotherapeutics or radiation are likely to have wider applications in cancer therapy due to greater efficacy. Thus, increased efficacy has been demonstrated in refractory or recurrent CD20-positive B-cell lymphoma patients receiving rituximab plus chemotherapy compared with those receiving chemotherapy alone.[182] Likewise, trastuzumab combined with paclitaxel was found to provide a greater response than paclitaxel alone. Such a combined-therapy approach with anti-EGFR MAbs might also allow for a reduction in the dose of chemotherapy or radiation needed to produce a given antitumor effect, decreasing the occurrence or severity of adverse events. Phase I and II trials exploring the safety and optimal administration of these combinations have shown promising activity, and phase III trials are underway.

The ability of EGFR inhibitors to sensitize tumor cells to radiation or chemotherapy has important implications for treatment of EGFR-overexpressing tumors. This is especially true for tumors that are commonly treated with radiation therapy, such as breast and head and neck cancers, for which locoregional control with standard therapy is poor in patients with locally advanced disease. The interesting preclinical data using combination radiation therapy and chemotherapy plus EGFR inhibitors will need to be substantiated through clinical trials, which are in progress.

These exciting results demonstrate that our increasing understanding of cancer biology is beginning to allow for a more targeted approach for cancer therapeutics. Over the last several years, studies of the HER2 oncogene and its overexpression in tumors have led to the successful dev l-opment of trastuzumab for treatment of HER2-overexpressing metastatic breast cancer. Similarly, detailed preclinical studies of the EGFR, including its ligands, EGFR overexpression in tumor cells, signal transduction pathways, and inhibition by specific MAbs and tyrosine kinase inhibitors, may provide effective, targeted therapeutics for EGFR-positive cancers.

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